Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

H_HVEM Reporter Jurkat Cell Line

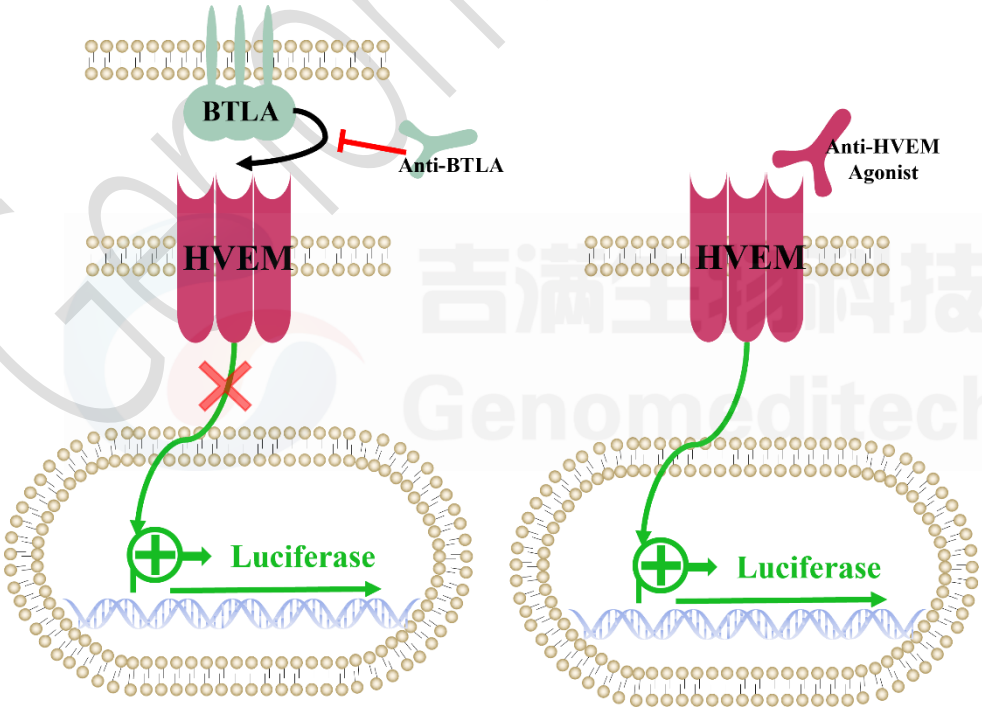
Catalog number: GM-C25497

Version 3.3.1.241129

HVEM (TNFRSF14) is a member of the tumor necrosis factor (TNF) receptor family and is a transmembrane protein that structurally consists of multiple extracellular cysteine-rich domains (CRDs). HVEM is widely expressed on various immune cells, including dendritic cells, naive T cells, B cells, NK cells, monocytes, and neutrophils.

HVEM has several ligands, such as BTLA, CD160, LIGHT, and lymphotoxin-α (LTα). The BTLA signaling pathway is activated through the interaction with HVEM. This binding reduces T cell activation and cytokine secretion by inhibiting downstream pathways like NF-κB and MAPK. This regulation prevents excessive immune activation and autoimmune diseases. BTLA can also interact with other ligands, further affecting immune cell function, making it valuable for research in immune regulation and cancer immunotherapy.

H_HVEM Reporter Jurkat Cell Line is a clonal stable Jurkat cell line constructed using lentiviral technology, constitutively expressing the HVEM, along with signal-dependent expression of a luciferase reporter gene. The binding of the agonistic antibodies or BTLA to HVEM (TNFRSF14) activates downstream reporter genes, leading to luciferase expression. Blockade antibodies of BTLA can inhibit BTLA-HVEM signal transmission. The luciferase readout represents the activation level of the signaling pathway and can thus be used for evaluating the in vitro effects of related drugs of BTLA or HVEM.



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Specifications

Quantity	5E6 Cells per vial,1 mL
Product Format	1 vial of frozen cells
Shipping	Shipped on dry ice
Storage Conditions	Liquid nitrogen immediately upon receipt
Recovery Medium	RPMI 1640+10% FBS+1% P.S
Growth medium	RPMI 1640+10% FBS+1% P.S+3.5 μg/mL Blasticidin+0.75 μg/mL Puromycin
Note	None
Freezing Medium	90% FBS+10% DMSO
Growth properties	Suspension
Growth Conditions	37°C, 5% CO_2
Mycoplasma Testing	The cell line has been screened to confirm the absence of Mycoplasma species.
Safety considerations	Biosafety Level 2
Note	It is recommended to expand the cell culture and store a minimum of 10 vials at an early passage for potential future use.

Materials

Reagent	Manufacturer/Catalogue No.
RPMI 1640	gibco/C11875500BT
Fetal Bovine Serum	Cegrogen biotech/A0500-3010
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/GM-040404
Puromycin	Genomeditech/GM-040401
H_BTLA CHO-K1 Cell Line	Genomeditech/GM-C19148
Anti-TNFRSF14(HVEM) hIgG4 Antibody	Genomeditech/GM-49928AB
Anti-BTLA hIgG4 Antibody(22B3)	Genomeditech/GM-50103AB
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040503

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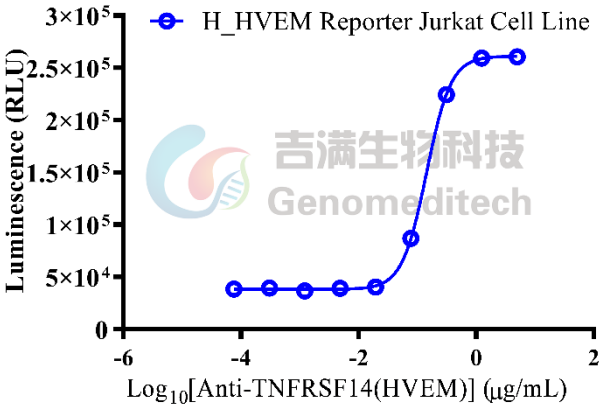
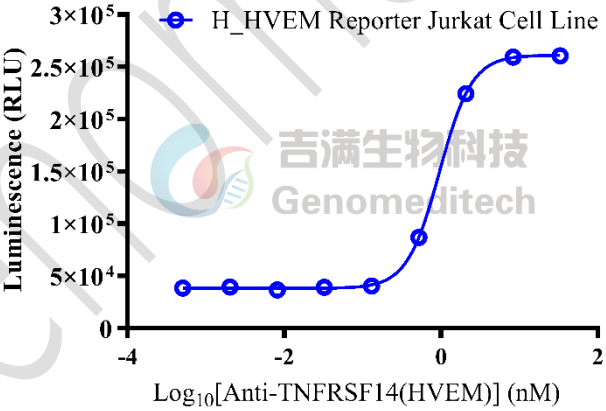
Figures



	H_HVEM Reporter Jurkat Cell Line
EC50	0.1438

Figure 1 | Response to Anti-TNFRSF14(HVEM) hIgG4 Antibody. The H_HVEM Reporter Jurkat Cell Line (Cat. GM-C25497) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Anti-TNFRSF14(HVEM) hIgG4 Antibody (Cat. GM-49928AB) in assay buffer (RPMI 1640 + 1% FBS + 1% P.S) for 16 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [6.0]. Data are shown by drug mass concentration.



	H_HVEM Reporter Jurkat Cell Line
EC50	0.9589

Figure 2 | Response to Anti-TNFRSF14(HVEM) hIgG4 Antibody. The H_HVEM Reporter Jurkat Cell Line (Cat. GM-C25497) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Anti-TNFRSF14(HVEM) hIgG4 Antibody (Cat. GM-49928AB) in assay buffer (RPMI 1640 + 1% FBS + 1% P.S) for 16 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [6.0]. Data are shown by drug molar concentration.

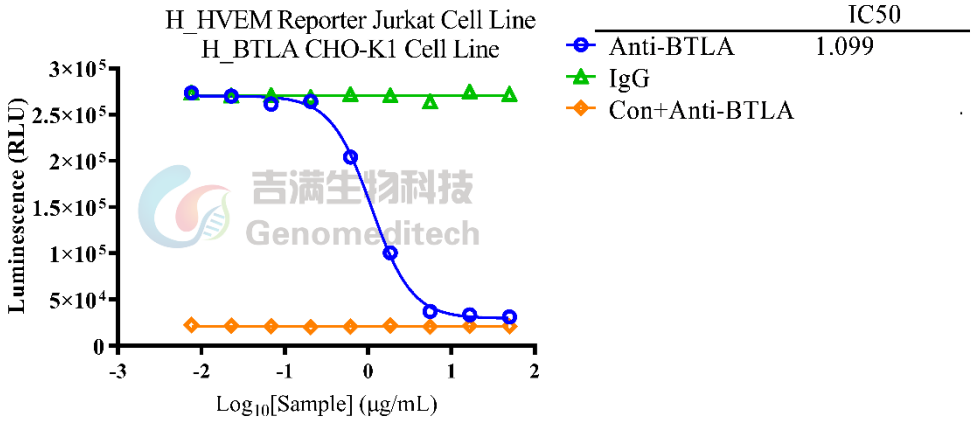


Figure 3 | Response to Anti-BTLA hIgG4 Antibody(22B3). Serial dilutions of the Anti-BTLA hIgG4 Antibody(22B3) were incubated with 1E4 cells/well of the H_BTLA CHO-K1 Cell Line (Cat. GM-C19148) in a 96-well plate. Subsequently, the H_HVEM Reporter Jurkat Cell Line (Cat. GM-C25497) at a concentration of 1E5 cells/well was added, and the co-culture proceeded for 7 hours in assay buffer (RPMI 1640 + 1% FBS + 1% P.S). Firefly luciferase activity is then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The results indicated a maximum blocking fold of approximately [8.9]. Data are shown by drug mass concentration. The other two experiments serve as controls.

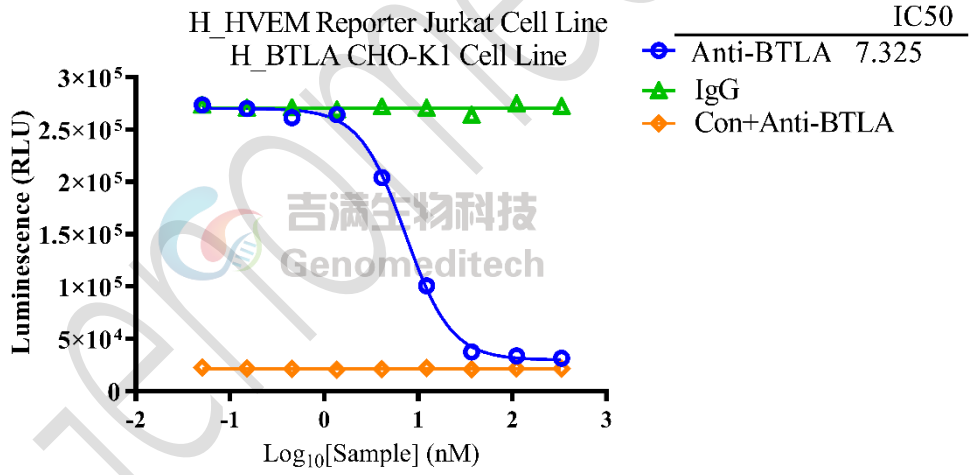
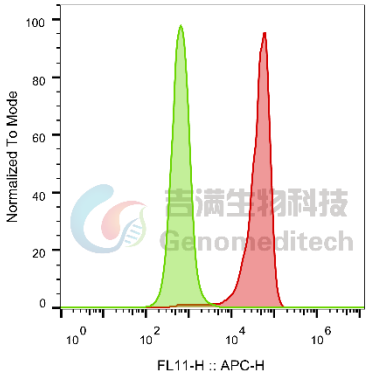


Figure 4 | Response to Anti-BTLA hIgG4 Antibody(22B3). Serial dilutions of the Anti-BTLA hIgG4 Antibody(22B3) were incubated with 1E4 cells/well of the H_BTLA CHO-K1 Cell Line (Cat. GM-C19148) in a 96-well plate. Subsequently, the H_HVEM Reporter Jurkat Cell Line (Cat. GM-C25497) at a concentration of 1E5 cells/well was added, and the co-culture proceeded for 7 hours in assay buffer (RPMI 1640 + 1% FBS + 1% P.S). Firefly luciferase activity is then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The results indicated a maximum blocking fold of approximately [8.9]. Data are shown by drug molar concentration. The other two experiments serve as controls.



SampleID	Geometric Mean : FL11-H
H_HVEM Reporter Jurkat H_IgG+APC-2nd Ab	662
H_HVEM Reporter Jurkat anti-TNFRSF14(HVEM)+APC-2nd Ab	41037

Figure 5 | H_HVEM Reporter Jurkat Cell Line (Cat. GM-C25497) was determined by flow cytometry using Anti-TNFRSF14(HVEM) hIgG4 Antibody (Cat. GM-49928AB).

Cell Recovery

Recovery Medium: RPMI 1640+10% FBS+1% P.S

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

a) Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 - 3 minutes).

b) Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.

c) Transfer the vial contents to a centrifuge tube containing 5.0 mL complete culture medium. And spin at approximately 176 x g for 5 minutes. Discard supernatant.

d) Resuspend cell pellet with the recommended complete medium. And dispense the suspension into 1 - 2 T-25 culture flasks.

e) Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Cell Freezing

Freezing Medium: 90% FBS+10% DMSO

a) Centrifuge at 176 x g for 3 minutes to collect cells.

b) Resuspend the cells in pre-cooled freezing medium and adjust the cell density to 5E6 cells/mL.

c) Aliquot 1 mL into each vial.

d) Place the vial in a controlled-rate freezing container and store at -80°C for at least 1 day, then transfer to liquid nitrogen as soon as possible.

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Cell passage

Growth medium: RPMI 1640+10% FBS+1% P.S+3.5 μg/mL Blasticidin+0.75 μg/mL Puromycin

Approximately 48-72 hours after the initial thawing, the cells can be passaged for the first time. After this initial passage, the culture medium can be adjusted to growth medium supplemented with antibiotics. If cells are not passaged within 48 hours, it is recommended to add some fresh recovery medium and place the flask horizontally.

a) When the cell density reaches 1.5 - 2E6 cells/mL, subculture the cells. Do not allow the cell density to exceed 2E6 cells/mL.

b) It is recommended to use T-25 flasks for subculturing.

c) These cells are suspension cells, and it is recommended to use the "half-medium change" method to maintain optimal cell conditions during passaging.

d) During passaging, you can directly add fresh growth medium to the culture flask, gently pipette to resuspend the cells, and then transfer the cell suspension to a new T-25 flask for continued culture.

Subcultivation Ratio: Maintain cultures at a cell concentraion between 3E5 and 1E6 viable cells/mL.

Medium Renewal: Every 2 to 3 days

Notes

a) These cells are sensitive to density, so please ensure that the cell density is maintained within an appropriate range during culture and subculturing.

b) During the first passage, pay attention to the nutrient supply; if not subculturing, make sure to add fresh recovery medium every other day as needed.

Related Products

BTLA:HVEM:LIGHT	
H_BTLA PD-1 Reporter Cell Line	H_BTLA Reporter Cell Line
H_HVEM aAPC CHO-K1 Cell Line	H_HVEM PD-L1 aAPC CHO-K1 Cell Line
Cynomolgus_BTLA HEK-293 Cell Line	H_BTLA CHO-K1 Cell Line
H_BTLA HEK-293 Cell Line	H_LIGHT(TNFSF14) CHO-K1 Cell Line
H_TNFRSF14(HVEM) CHO-K1 Cell Line	
Anti-BTLA hIgG4 Antibody(22B3)	Anti-BTLA hIgG4 Antibody(Icatolimab)
Anti-TNFRSF14(HVEM) hIgG4 Antibody	Anti-TNFSF14 hIgG4 Antibody

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